

## **28. Abstract**

The present invention relates to a method for the detection of the methylation status of a nucleotide at a predetermined position in a nucleic acid molecule comprising the steps of (a) treating a sample comprising said nucleic acid molecule or consisting of said nucleic acid molecule in an aqueous solution with an agent suitable for the conversion of said nucleotide if present in (i) methylated form; or (ii) non-methylated form to pair with a nucleotide normally not pairing with said nucleotide prior to conversion; (b) amplifying said nucleic acid molecule treated with said agent; (c) real-time sequencing said amplified nucleic acid molecule; and (d) detecting whether said nucleotide is formerly methylated or not methylated in said predetermined position in the sample. The invention further relates to a method for the diagnosis of a pathological condition or the predisposition for a pathological condition comprising detection of a methylation status nucleotide at a predetermined position in a nucleic acid molecule comprising the steps of (a) treating a sample comprising said nucleic acid molecule or consisting of said nucleic acid molecule in an aqueous solution with an agent suitable for the conversion of said nucleotide if present in (i) methylated form; or (ii) non-methylated form to pair with a nucleotide normally not pairing with said nucleotide prior to conversion; (b) amplifying said nucleic acid molecule treated with said agent; (c) real-time sequencing said amplified nucleic acid molecule; and (d) detecting whether said nucleotide is formerly methylated or not methylated in said predetermined position in the sample wherein a methylated or not methylated nucleotide is indicative of a pathological condition or the predisposition for said pathological condition.